Reactive Oxygen Species in Living Systems: Source, Biochemistry, and Role in Human Disease

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Reactive oxygen species are constantly formed in the human body and removed by antioxidant defenses. An antioxidant is a substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants can act by scavenging biologically important reactive oxygen species ($O_2^- \cdot$, H_2O_2, $\cdot OH$, HOCl, ferryl, peroxyl, and alkoxyl), by preventing their formation, or by repairing the damage that they do. One problem with scavenging-type antioxidants is that secondary radicals derived from them can often themselves do biologic damage. These various principles will be illustrated by considering several thiol compounds.

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It is difficult these days to open a medical journal and not find some paper on the role of "reactive oxygen species" or "free radicals" in human disease. These species have been implicated in over 50 diseases [1]. This large number suggests that radicals are not something esoteric, but that they participate as a fundamental component of tissue injury in most, if not all, human diseases.

What are "free radicals" and "reactive oxygen species"? Do they cause disease? Are they produced in increased amounts as a result of disease and then contribute to further tissue injury? Are they merely an epiphenomenon of no relevance to clinical medicine? This introductory article attempts to answer such questions.

WHAT IS A FREE RADICAL?

Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of two electrons, spinning in opposite directions. A free radical can be defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. Most biologic molecules are nonradicals, containing only paired electrons.

An electron occupying an orbital by itself has two possible directions of spin. Indeed, the technique of measuring electron spin resonance detects radicals by measuring the energy changes that occur as unpaired electrons 'relax' following alignment in response to a magnetic field [2]. Since electrons are more stable when paired together in orbitals, radicals generally are more reactive than nonradicals, although there is a considerable variation in their reactivity.

Radicals can react with other molecules in a number of ways [3]. If two radicals meet, they can combine their unpaired electrons (symbolized by $\cdot$) and join to form a covalent bond (a shared pair of electrons). The hydrogen atom, with one unpaired electron, is a radical and two atoms of hydrogen easily combine to form the diatomic hydrogen molecule:

$$H\cdot + H\cdot \longrightarrow H_2 \qquad (1)$$

Radicals react with nonradicals in several ways. A radical may donate its unpaired electron to a nonradical (a reducing radical) or it might take an electron from another molecule in order to form a pair (an oxidizing radical). A radical may also join onto a nonradical. Whichever of these three types of reaction occurs, the nonradical species becomes a radical. A feature of the reactions of free radicals with nonradicals is that they tend to proceed as chain reactions, where one radical begets another.

For many years, chemists have been interested in free radical reactions. Many plastics, such as polythene, arise by free radical chain polymerization [4]. Combustion is a free radical reaction. The drying and aging of paint also involves free radical reactions. Curators of museums have studied the role of free radical damage in the age-dependent deterioration of paintings and other items [5]. Metabolism of toxins in the human body can produce radicals. For example, carbon tetrachloride (CCl_4) is metabolized in the endoplasmic reticulum of the liver to produce the damaging trichloromethyl radical, $CCl_3 \cdot$ [3].

HYDROXYL RADICAL

Chemists and biologists have examined in detail the role of free radical reactions in the damage done to living cells by high-energy radiation. When tissues are exposed to, for example, gamma radiation, most of the energy taken up is absorbed by the cell water, largely because there is more water there than any other molecule. The radiation causes one of the oxygen–hydrogen covalent bonds in water to split, leaving a single electron on hydrogen and one on oxygen, thus creating two radicals:

$$H{-}O{-}H \xrightarrow[\text{stages}]{\text{intermediate}} H\cdot + \cdot OH \qquad (2)$$

$H\cdot$ is a hydrogen radical (or hydrogen atom), and $\cdot OH$ is a hydroxyl radical. The latter is the most reactive radical known to chemistry. It can attack and damage almost every molecule found in living cells at a diffusion-controlled rate, i.e., $\cdot OH$ reacts as soon as it comes into contact with another molecule in solution. Since it is so reactive, $\cdot OH$ generated in vivo does not persist for even a microsecond and rapidly combines with molecules in its immediate vicinity.

Reactions of $\cdot OH$ with biologic molecules, most of which are nonradicals, set off chain reactions [1]. Reactions of $\cdot OH$ include its ability to interact with the purine and pyrimidine bases of DNA, leading to radicals that have a number of possible chemical fates [6]. $\cdot OH$ can also abstract hydrogen atoms from many biologic molecules, including thiols:

$$R{-}SH + \cdot OH \longrightarrow RS\cdot + H_2O \qquad (3)$$

The resulting sulfur radicals (thiyl radicals) have many interesting chemical properties. They can combine with oxygen to generate oxysulfur radicals, such as $RSO_2\cdot$ and $RSO\cdot$, a number of which damage biologic molecules [7–9]. For example, sulfur-containing radicals derived from the drug penicillamine are able to attack and damage certain proteins [10]. When discussing the use of thiol compounds as free radical scavengers, it is essential to ask what may happen to the resulting sulfur radicals in biologic systems [11].

Perhaps the best-characterized biologic damage caused by $\cdot OH$ is its ability to stimulate the free radical chain reaction known as lipid peroxidation. This occurs when the $\cdot OH$ is generated close to membranes and attacks the fatty acid side chains of the membrane phospholipids. It preferentially attacks polyunsaturated fatty acid side chains, such as arachidonic acid. The $\cdot OH$ abstracts an atom of hydrogen from one of the carbon atoms in the side chain and combines with it to form water:

$$-CH- + \cdot OH \longrightarrow -\dot{C}- + H_2O \qquad (4)$$

Reaction (4) removes the $\cdot OH$, but leaves behind a carbon-centered radical (—Ċ—) in the membrane. Carbon-centered radicals formed from polyunsaturated fatty acid side chains usually undergo molecular rearrangement to give conjugated diene structures, which can have various fates. Thus, if two such radicals collided in the membrane, cross-linking of fatty acid side chains could occur as the two electrons joined to form a covalent bond. Reaction with membrane proteins is also a possibility. However, under physiologic conditions, the most likely fate of carbon-centered radicals is to combine with oxygen, creating yet another radical, the peroxyl radical (sometimes abbreviated to the peroxy radical):

$$-\dot{C}- + O_2 \longrightarrow -\overset{\displaystyle O_2}{\overset{|}{C}}- \qquad (5)$$

Peroxyl radicals are reactive enough to attack adjacent fatty acid side chains, abstracting hydrogen:

$$-\overset{\displaystyle O_2}{\overset{|}{C}}- + -\overset{\displaystyle H}{\overset{|}{C}}- \longrightarrow -\overset{\displaystyle O_2H}{\overset{|}{C}}- + -\dot{C}-$$

lipid hydroperoxide (6)

Another carbon-centered radical is generated, and so the chain reaction [equations (5) and (6)] con-

tinues. One ·OH can result in the conversion of many hundred fatty acid side chains into lipid hydroperoxides. Accumulation of lipid hydroperoxides in a membrane disrupts its function and can cause it to collapse. Lipid hydroperoxides can also decompose to yield a range of highly cytotoxic products, among the most unpleasant of which are aldehydes [12]. A great deal of attention in the literature has been focused on malonaldehyde (malondialdehyde), but this is much less noxious than such products as 4-hydroxynonenal [12,13]. Peroxyl radicals and cytotoxic aldehydes can also cause severe damage to membrane proteins, inactivating receptors and membrane-bound enzymes [14].

SOURCES OF OXYGEN RADICALS IN VIVO

Biochemists (apart from those with a special interest in "background" free radical generation in vivo, due to exposure to ionizing radiation) became interested in radicals only in the 1970s. This interest followed from the discovery in 1968 of superoxide dismutase (SOD), an enzyme specific for a free radical substrate [15]. SOD removes superoxide radical, a species that is formed by adding an extra electron onto the oxygen molecule:

$$O_2 + e^- \longrightarrow \underset{\text{superoxide radical}}{O_2^- \cdot} \qquad (7)$$

SOD removes $O_2^- \cdot$ by catalyzing a dismutation reaction, involving oxidation of one $O_2^- \cdot$ to oxygen and reduction of another $O_2^- \cdot$ to hydrogen peroxide:

$$O_2^- \cdot + O_2^- \cdot + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad (8)$$

In the absence of SOD, reaction (8) occurs nonenzymically but at a rate approximately four orders of magnitude less at pH 7.4.

The discovery of SOD led to the realization that $O_2^- \cdot$ is formed in vivo in living organisms, and SOD removes it. Some of the $O_2^- \cdot$ formed in vivo arises from a chemical accident. For example, when mitochondria are functioning, some of the electrons passing through the respiratory chain leak from the electron carriers and pass directly onto oxygen, reducing it to $O_2^- \cdot$ [15,16]. Many molecules oxidize on contact with oxygen, e.g., an epinephrine solution left on the bench "goes off" and eventually forms a pink product. The first stage in this oxidation is transfer of an electron from the epinephrine to O_2, forming $O_2^- \cdot$. Such oxidations undoubtedly proceed in vivo as well [1]. For example, several sugars, including glucose, interact with proteins to produce oxygen radicals. It has been suggested that decades of exposure of body tissues to elevated blood glucose can result in diabetic patients suffering "oxidative stress" that may contribute to the side effects of hyperglycemia [17]. Glycation of proteins involves not only direct reaction with the sugar but also free radical reactions [17].

Thiols can also be oxidized in the presence of oxygen, generating sulfur-containing radicals as well as $O_2^- \cdot$ and ·OH. Thiol oxidation is favored by alkaline pH values and by the presence of transition metal ions, especially copper ions [18]. Thus, mixtures of copper ions and thiols can be cytotoxic, as shown for cysteine [19]. Iron ions can also promote free radical generation from thiols under certain circumstances [20]. Attempts to use thiols as antioxidants in systems containing iron or copper ions may even result in stimulation of oxidative damage.

Superoxide and Phagocyte Action

Some of the $O_2^- \cdot$ production in vivo may be accidental but much is functional. Activated phagocytic cells generate $O_2^- \cdot$ as shown for monocytes, neutrophils, eosinophils, and macrophages of all types [21]. Radical production is important in allowing phagocytes to kill some of the bacterial strains that they engulf. This can be illustrated by examining patients with chronic granulomatous disease, a series of inborn conditions in which the membrane-bound reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in phagocytes that makes the $O_2^- \cdot$ fails to work [21]. Such patients have phagocytes that engulf and process bacteria normally, but several bacterial strains are not killed and are released in viable form when the phagocytes die. Thus, patients suffer severe, persistent, and multiple infections with such organisms as *Staphylococcus aureus*. Another killing mechanism used by neutrophils (but not by macrophages) is the enzyme myeloperoxidase [22]. It uses H_2O_2 produced by dismutation of $O_2^- \cdot$ to oxidize chloride ions into hypochlorous acid (HOCl), a powerful antibacterial agent:

$$H_2O_2 + Cl^- \longrightarrow HOCl + OH^- \qquad (9)$$

Thiol groups are easily oxidized by HOCl. Hence, low molecular mass thiol compounds such as glutathione (GSH), N-acetylcysteine, and mercaptopropionylglycine are very effective at protecting, for example, proteins against oxidative damage by HOCl [23,24].

Superoxide formed in vivo, whether functionally or accidentally, is disposed of by SOD [equation (8)]. Recent studies using genetic engineering techniques to manipulate SOD levels of organisms, or to delete the genes encoding SOD, provide further

evidence of the importance of SOD [25]. It is interesting to note that no complete inborn deficiencies of SOD have been reported in humans, perhaps because they would be lethal mutations.

Reactive Oxygen Species

SOD removes $O_2^- \cdot$ by converting it into hydrogen peroxide (H_2O_2) and O_2 [equation (8)]. H_2O_2 itself can be quite toxic to cells. For example, incubation of cells with H_2O_2 causes deoxyribonucleic acid (DNA) damage, membrane disruption, and release of Ca^{2+} ions within the cells, leading to activation of Ca^{2+}-dependent proteases and nucleases [26]. At least some of this damage may be mediated by a reaction of H_2O_2 with $O_2^- \cdot$ in the presence of iron or copper ions, to form highly reactive radicals, one of which is $\cdot OH$. This reaction proceeds in a number of stages, but the overall process is summarized by

$$O_2^- \cdot + H_2O_2 \xrightarrow{\text{Fe or Cu ions}} \underset{\text{hydroxyl radical}}{\cdot OH} + OH^- + O_2 \quad (10)$$

Thus, removal of H_2O_2, as well as of $O_2^- \cdot$, is biologically advantageous [27].

SOD therefore works in conjunction with two enzymes, catalase and glutathione peroxidase [27], that remove H_2O_2 in human cells. The study of inborn errors of metabolism suggests that glutathione peroxidase (GSH–Px) is the more important of the two in removing H_2O_2, probably because it is located in the same subcellular compartments (cytosol and mitochondria) as SOD. GSH–Px has the distinction of being the only human enzyme known requiring the element selenium for its activity; a selenocysteine residue (side chain –SeH instead of –SH, as in normal cysteine) is present at its active site. However, it is unlikely that the sole function of selenium in humans is to act as a cofactor for GSH–Px [28]. GSH–Px removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG):

$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O \quad (11)$$

H_2O_2 has no unpaired electrons and does not qualify as a radical. Hence, the term reactive oxygen species has been introduced to describe collectively not only $O_2^- \cdot$ and $\cdot OH$ (radicals) but also H_2O_2 (nonradical). Hypochlorous acid (HOCl) produced by myeloperoxidase is also a nonradical, having no unpaired electrons. H_2O_2, $O_2^- \cdot$, $\cdot OH$, and HOCl are sometimes collectively called "oxidants." This is a valid description of H_2O_2, $\cdot OH$, and HOCl, which are oxidizing agents. However, $O_2^- \cdot$ has both oxidizing and reducing properties. The latter property is used in a popular assay for $O_2^- \cdot$, the SOD-inhibitable reduction of cytochrome c, often applied to measure $O_2^- \cdot$ production by phagocytes:

$$\text{cyt c } (Fe^{3+}) + O_2^- \cdot \longrightarrow O_2 + \text{cyt c } (Fe^{2+}) \quad (12)$$

TRANSITION METAL IONS AND FREE RADICAL REACTIONS

Many transition metals have variable oxidation numbers, e.g., iron has Fe^{2+} and Fe^{3+} ions and copper has Cu^+ and Cu^{2+} ions. Changing between oxidation states involves accepting and donating single electrons, e.g.,

$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+} \quad (13)$$

$$Cu^{2+} + e^- \rightleftharpoons Cu^+ \quad (14)$$

Transition metal ions are remarkably good promoters of free radical reactions [29]. Polymer scientists and food chemists have been aware of this for years [4,30], and biochemists are learning it too [1,17–20,26,31–34]. It has already been noted that copper ions promote oxidation of thiols:

$$R\text{–}SH + Cu^{2+} \longrightarrow R\text{–}S \cdot + Cu^+ + H^+ \quad (15)$$

and that Fe^{2+} ions reduce H_2O_2 to give $\cdot OH$ [equation (10)].

Transition Metals and Lipid Peroxidation

Transition metal ions are involved in lipid peroxidation in two ways. They can participate in first-chain initiation, which involves attack by any species capable of abstracting a hydrogen atom. $\cdot OH$, which has this property, is produced by the reaction of $O_2^- \cdot$ and H_2O_2 with iron ion catalysis [equation (10)]. It is also be produced by reaction of H_2O_2 with copper ions, probably in addition to oxidizing copper(III)–oxygen complexes [26,31]. Several iron ion–oxygen complexes, such as perferryl, ferryl, or $Fe^{2+}/Fe^{3+}/O_2$ complexes, have also been claimed to initiate peroxidation [32], although their ability to do so is uncertain [33,34].

Transition metal ions also affect lipid peroxidation by decomposing peroxides. Commercial fatty acids are heavily contaminated with peroxides [34]. Cell disruption to isolate membrane fractions increases rates of nonenzymic free radical reactions and activates enzymes (cyclooxygenases and lipooxygenases) that produce peroxides (**Figure 1**). When transition metal ions are added to lipid systems already containing peroxides, their main action is to decompose these peroxides into peroxyl and alkoxyl (lipid–O $\cdot$) radicals that in turn abstract

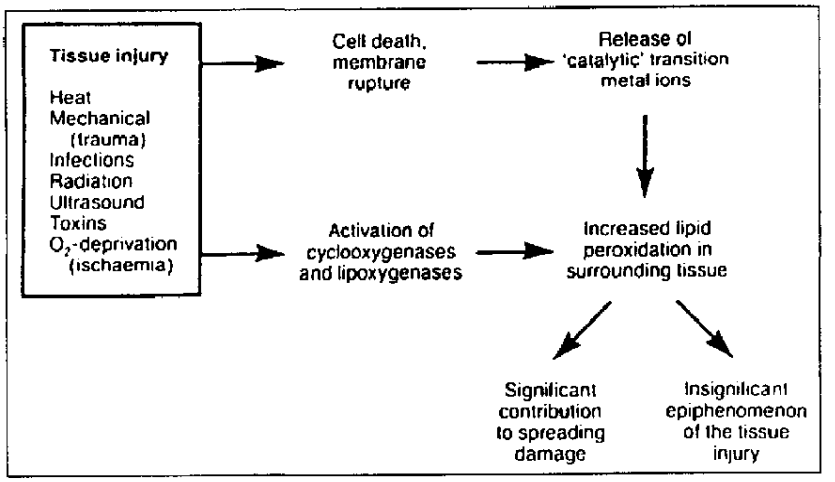

Figure 1. Diagram showing how cell injury can lead to increased free radical reactions in the surrounding area.

hydrogen and perpetuate the chain reaction of lipid peroxidation [34]. This may be represented by the following simplified equations, in which lipid · symbolises a carbon-centered radical

$$\text{lipid–OOH} + \underset{(Cu^{+})}{Fe^{2+}} \longrightarrow \underset{\text{alkoxyl radical}}{\text{lipid–O}\cdot} + \underset{(Cu^{2+})}{Fe^{3+}} + OH^{-} \quad (16)$$

$$\text{lipid–OOH} + Fe^{3+} \longrightarrow \underset{\text{peroxyl radical}}{\text{lipid–OO}\cdot} + Fe^{2+} + H^{+} \quad (17)$$

$$\text{lipid O}\cdot + \text{lipid–H} \longrightarrow \text{lipid–OH} + \text{lipid}\cdot \quad (18)$$

$$\text{lipid–OO}\cdot + \text{lipid–H} \longrightarrow \text{lipid–OOH} + \text{lipid}\cdot \quad (19)$$

$$\text{lipid}\cdot + O_2 \longrightarrow \text{lipid–OO}\cdot \quad (20)$$

Reducing agents, such as ascorbic acid or $O_2^-\cdot$, accelerate these metal ion-dependent peroxidation reactions because Cu^+ and Fe^{2+} ions seem to react with peroxides faster than do Cu^{2+} and Fe^{3+}, respectively. The end products of these complex metal ion-catalyzed breakdowns of lipid hydroperoxides include the cytotoxic aldehydes mentioned previously (malonaldehyde, 4-hydroxynonenal), as well as hydrocarbon gases such as ethane and pentane [1]. Some thiol compounds can also reduce metal ions and accelerate peroxidation of lipids, e.g., cysteine [35]. It has been suggested that some thiyl radicals (RS ·) initiate peroxidation by abstracting hydrogen atoms from lipids [36]. Different thiols behave differently in peroxidizing lipid systems, presumably depending on their metal ion-reducing ability and the reactivity of their thiyl radicals.

ANTIOXIDANT DEFENSE

Organisms use superoxide dismutases, catalase, and glutathione peroxidase as protection against generation of reactive oxygen species. Organisms also keep as many iron and copper ions as possible safely bound in storage or transport proteins [37–39]. There is three times as much transferrin iron-binding capacity in plasma as iron needing to be transported, so that there are essentially no free iron ions in the plasma [38]. Iron ions bound to transferrin cannot stimulate lipid peroxidation or formation of free · OH radicals. The same is true of copper ions bound to the plasma proteins ceruloplasmin or albumin [37–40]. The value of this sequestration is shown by an inspection of the pathology suffered by patients with iron-overload disease, in whom iron ion–citrate chelates circulate in the blood [40]. These patients can suffer liver damage, diabetes, joint inflammation, and hepatoma, among other problems [41]. Metal ion sequestration is an important antioxidant defense. For example, recent papers have referred to ascorbic acid as a major antioxidant in plasma. However, ascorbate can only exert antioxidant properties in the absence of transition metal ions [11].

Tocopherol

As well as the primary defenses (scavenger enzymes and metal-ion sequestration), secondary defenses are also present. The cell membranes and plasma lipoproteins contain α-tocopherol, a lipid-soluble molecule that functions as a chain-breaking antioxidant. Attached to the hydrophobic structure of α-tocopherol is an –OH group whose hydrogen atom is easily removed. Hence, peroxyl and alkoxyl radicals generated during lipid peroxidation preferentially combine with the antioxidant, e.g.,

$$\text{tocopherol–OH} + \text{—}\overset{O_2}{\overset{|}{C}}\text{—} \longrightarrow \text{tocopherol–O}\cdot + \text{—}\overset{O_2H}{\overset{|}{C}}\text{—} \quad (21)$$

instead of with an adjacent fatty acid side chain. This therefore terminates the chain reaction, whence the term chain-breaking antioxidant. It also converts the α-tocopherol into a new radical, tocopherol–O ·, which is poorly reactive and unable to attack adjacent fatty acid side chains, consequently stopping the chain reaction. Evidence exists [43,44] that the tocopherol radical can migrate to the membrane surface and reconvert to α-tocopherol by reaction with ascorbic acid (vitamin C). Both vitamin C and α-tocopherol seem to minimize the consequences of lipid peroxidation in lipoproteins and in membranes, should this process begin. Some thiol compounds, such as GSH, might also be involved in regenerating α-tocopherol from its radical in vivo [44].

The terms "α-tocopherol" and "vitamin E" are often used synonymously, which is not strictly correct. Vitamin E is defined nutritionally as a factor needed in the diet of pregnant female rats to prevent resorption of the fetus [45] and compounds other than α-tocopherol (e.g., β-, γ-, and δ-tocopherols) have some effect in this assay. However, α-tocopherol is the most effective, and it seems to be the most important lipid-soluble chain-breaking antioxidant in vivo in humans [46]. The content of α-tocopherol in circulating low-density lipoproteins helps to determine their resistance to lipid peroxidation and thus may affect the development of atherosclerosis, a disease in which lipid peroxidation is involved [47]. Low plasma levels of α-tocopherol and vitamin C correlate with an increased incidence of myocardial infarction and of some forms of cancer [47].

Other Antioxidants and Repair Systems

Some other compounds may also function as antioxidants in vivo, such as uric acid, ubiquinol, and bilirubin (reviewed in [11]). Antioxidant defenses are not quite perfect. Cells contain systems that can repair DNA after attack by radicals [48], degrade proteins damaged by radicals [49], and metabolize lipid hydroperoxides [1].

WHAT CAN WE EXPECT FROM ANTIOXIDANTS IN THE THERAPY OF HUMAN DISEASE?

What Is an Antioxidant?

"Antioxidant" can be defined in various ways. Often, the term is implicitly restricted to chain-breaking antioxidant inhibitors of lipid peroxidation, such as vitamin E. However, the author prefers a broader definition—an antioxidant is any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [1]. The term "oxidizable substrate" includes almost everything found in living cells, including proteins, lipids, carbohydrates, and DNA.

TABLE I

Questions to Ask When Evaluating the Proposed Role of an "Antioxidant" In Vivo

1. What biomolecule is the compound supposed to protect? An inhibitor of lipid peroxidation is unlikely to be useful if the oxidative damage is mediated by an attack on proteins or DNA.
2. Is the compound present in vivo at or near that biomolecule at sufficient concentration? For example, many compounds have been suggested to act as · OH scavengers in vivo. In order to compete with biologic molecules for · OH, a scavenger must be present in at least millimolar concentrations in vivo. Most drugs never achieve this sort of concentration.
3. How does it protect: by scavenging reactive oxygen species, by preventing their formation, or by repairing damage?
4. For naturally occurring antioxidants, is antioxidant protection the primary biologic role of the molecule or a secondary one? For example, SOD has probably evolved as an antioxidant enzyme. By contrast, transferrin has probably evolved as an iron transport protein, although the binding of iron ions to transferrin prevents them from accelerating radical reactions, giving this protein an important secondary role in extracellular antioxidant defense.
5. If the antioxidant acts by scavenging a reactive oxygen species, can the antioxidant-derived radicals themselves do biologic damage?
6. Can the antioxidant cause damage in biologic systems different from those in which it exerts protection?

Antioxidants act in many different ways (**Table I**). In proposing antioxidants for use in human disease, it is important to note the following: (a) the precise role played in the disease pathology by reactive oxygen species; and (b) the molecular targets of oxidative damage that need protecting. Thus, oxidative stress can damage a multiplicity of targets in living cells and the initial damage to one target can then affect others [26]. **Figure 2** attempts to illustrate some of the complex interacting mechanisms by which excess production of reactive oxygen species can produce cell damage. If, for example, the primary event is damage to DNA, then an inhibitor of lipid peroxidation might offer little or no protection.

Free Radicals and Human Disease: Causation or Consequence?

Does increased formation of free radicals and other reactive oxygen species cause any human disease? Radiation-induced carcinogenesis may be initiated by free radical damage [48]. The signs produced by chronic dietary deficiencies of selenium (Keshan disease) or of vitamin E (neurologic disorders seen in patients with inborn errors in the mechanism of intestinal fat absorption) could also be mediated by reactive oxygen species [28,50]. In the premature infant, exposure of the incompletely vascularized retina to elevated concentrations of oxygen can lead to retinopathy of prematurity, which in its most severe forms can result in blindness. Several controlled clinical trials have documented the efficiency of α-tocopherol in minimizing the retinopathy [51], suggesting a role for lipid peroxidation.

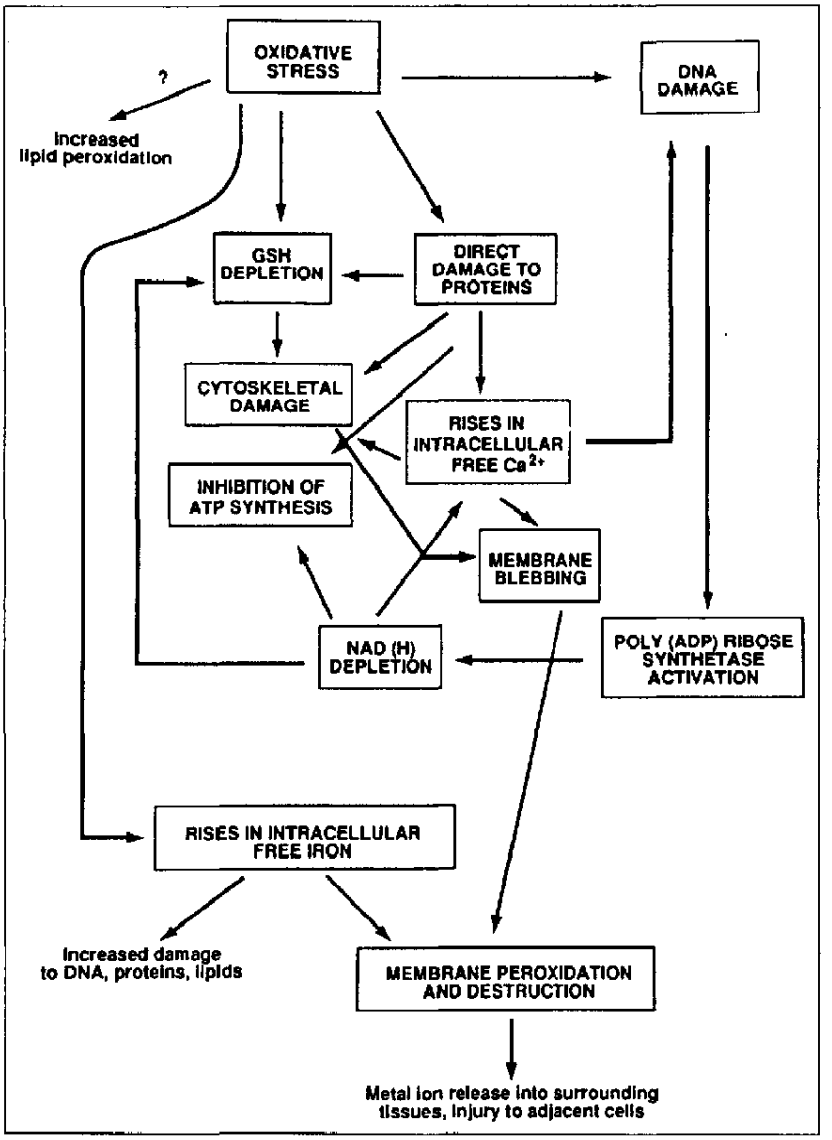

Figure 2. Diagram to illustrate the complex multifactorial nature of oxidative damage to cells.

For most human diseases, increased formation of reactive oxygen species is secondary to the primary disease process. For example, activated neutrophils produce $O_2^- \cdot$, H_2O_2, and HOCl in order to kill bacteria. If a large number of phagocytes become activated in a localized area, they can produce tissue damage. The synovial fluid in the swollen knee joints of rheumatoid patients swarm with activated neutrophils. There is evidence that reactive oxygen species and other products derived from neutrophils contribute to the joint injury. Whether this is a major or a minor contribution to joint damage remains to be established [52]. In some forms of adult respiratory distress syndrome (ARDS), lung damage seems to be mediated by an influx of neutrophils into the lung, where they become activated to produce prostaglandins, leukotrienes, proteolytic enzymes such as elastase, and reactive oxygen species [53]. Among other effects, reactive oxygen species inactivate proteins (such as α_1-antiproteinase) within the lung that normally inhibit the action of elastase and prevent it from attacking lung elastic fibers. The precise contribution of oxidative damage to lung injury in ARDS is unknown, but deserves investigation in view of the high mortality rate.

In both ARDS and in rheumatoid arthritis, increased generation of reactive oxygen species is secondary to the processes that cause neutrophil infiltration, but they then may make an additional detrimental contribution to tissue injury.

There are several examples in which injury, by a nonradical mechanism, leads to increased free radical reactions. Mechanical (e.g., crushing) or chemi-

cal injury to tissues can cause cells to rupture and release their contents, including transition metal ions (Figure 1), into the surrounding area. Administration of cytotoxic drugs to patients with acute myeloid leukemia has been shown to create a temporary "iron-overload" state, probably due to extensive drug-induced lysis of the leukemic cells. This increased iron availability could contribute to the side effects of cytotoxic chemotherapy [54].

Perhaps the greatest interest in this area lies in the sequelae of traumatic or ischemic injury to the brain. Some areas of the human brain are rich in iron. Cerebrospinal fluid has no significant iron-binding capacity, since its content of transferrin is low. It has been proposed [55] that injury to the brain by mechanical means (trauma) or by oxygen deprivation (stroke) can result in release of iron ions into the surrounding area. These ions facilitate further damage to the surrounding areas by accelerating free radical reactions. This proposal has been given some support from animal studies, using antioxidants such as chelating agents that bind iron ions and prevent them from catalyzing radical reactions. Promising results have been obtained with amino-steroid–based antioxidants. Thus, one such "lazaroid," U74006F, has been observed to decrease the effects of reperfusion injury upon the brain of cats [56] to decrease post-traumatic spinal cord degeneration in cats [57] and to minimize neurologic damage after head injury in mice [58].

Free Radicals in Human Disease: A Triviality?

Tissue destruction and degeneration can result in increased oxidative damage, by such processes as metal-ion release, phagocyte activation, lipoxygenase activation, and disruption of mitochondrial electron transport chains, so that more electrons "escape" to oxygen to form $O_2^-\cdot$ (Figure 1). It follows that almost any disease is likely to be accompanied by increased formation of reactive oxygen species. It is not therefore surprising that the list of diseases in which their formation has been implicated is long and is growing longer [1]. For atherosclerosis [43,59], rheumatoid arthritis [52], some forms of ARDS, reoxygenation injury [60,61], and traumatic or ischemic damage to the central nervous system, there is reasonable evidence to suggest that free radical reactions make a significant detrimental contribution to the pathologic process. As previously stressed [62], it is equally likely that in some (perhaps most) diseases, the increased ROS formation is an epiphenomenon, making no significant contribution to the progression of the disease. Each proposal must be subject to stringent examination, because the likely clinical value of "antioxidant therapy" will depend on how well the exact role of reactive oxygen species is known.

REFERENCES

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 1989.
2. Cammack R. Electron spin resonance. In: The biochemistry of plants, Vol. 13. New York: Academic Press, 1987:229–57.
3. Slater TF. Free radical mechanisms in tissue injury. Biochem J 1984; 222: 1–15.
4. Scott G. Potential toxicological problems associated with antioxidants in plastic and rubber consumables. Free Radic Res Commun 1988; 5: 141–7.
5. Daniels V. Oxidative damage and the preservation of organic artefacts. Free Radic Res Commun 1988; 5: 213–20.
6. Aruoma OI, Halliwell B, Dizdaroglu M. Iron ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. J Biol Chem 1989; 264: 13024–8.
7. Asmus KD. Sulphur-centered free radicals. In: Slater TF, ed. Radioprotectors and anticarcinogens. London: Academic Press, 1983: 23–42.
8. Sevilla MD, Yan M, Becker D, Gillich S. ESR investigations of the reactions of radiation-produced thiyl and DNA peroxyl radicals: formation of sulfoxyl radicals. Free Radic Res Commun 1989; 6: 21–4.
9. Monig J, Asmus KD, Forni LG, Willson RL. On the reaction of molecular oxygen with thiyl radicals: a re-examination. Int J Radiat Biol 1987; 52: 589–602.
10. Aruoma OI, Halliwell B, Butler J, Hoey BM. Apparent inactivation of α_1-antiproteinase by sulphur-containing radicals derived from penicillamine. Biochem Pharmacol 1989; 38: 4353–7.
11. Halliwell B. How to characterize a biologic antioxidant. Free Radic Res Commun 1990; 9: 569–71.
12. Esterbauer H, Zollner H, Schaur RJ. Hydroxyalkenals: cytotoxic products of lipid peroxidation. ISI Atlas Sci Biochem 1988; 1: 311–5.
13. Curzio M. Interaction between neutrophils and 4-hydroxyalkenals and consequences on neutrophil mobility. Free Radic Res Commun 1986; 5: 55–66.
14. Dean RT, Thomas SM, Garner A. Free-radical-mediated fragmentation of monoamine oxidase in the mitochondrial membrane. Role of lipid radicals. Biochem J 1986; 240: 489–94.
15. Fridovich I. Superoxide dismutases. Adv Enzymol 1974; 41: 35–48.
16. Fridovich I. Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol 1983; 23: 239–57.
17. Wolff SP, Dean RT. Glucose autoxidation and protein modification. The potential role of "autoxidative glycosylation" in diabetes. Biochem J 1987; 245: 243–50.
18. Albro PW, Corbett JT, Schroeder JL. Generation of hydrogen peroxide by incidental metal ion-catalyzed autoxidation of glutathione. J Inorg Biochem 1986; 27: 191–203.
19. Vina J, Saez GT, Wiggins D, Roberts AFC, Hems R, Krebs HA. The effect of cysteine oxidation on isolated hepatocytes. Biochem J 1983; 212: 39–44.
20. Rowley DA, Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of thiol compounds. FEBS Lett 1982; 138: 33–6.
21. Curnutte JT, Babior BM. Chronic granulomatous disease. Adv Hum Genet 1987; 16: 229–45.
22. Weiss SS. Tissue destruction by neutrophils. N Engl J Med 1989; 320: 365–76.
23. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine. Free Radic Biol Med 1989; 6: 593–7.
24. Puppo A, Cecchini R, Aruoma OI, Bolli R, Halliwell B. Scavenging of hypochlorous acid and of myoglobin-derived species by the cardioprotective agent mercaptopropionylglycine. Free Radic Res Commun 1990; 10: 371–81.
25. Farr SB, D'Ari R, Touati D. Oxygen-dependent mutagenesis in Escherichia coli lacking superoxide dismutase. Proc Natl Acad Sci USA 1986; 83: 8268–72.
26. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems.FEBS Lett 1991; 281: 9–19.
27. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979; 59: 527–605.
28. Levander OA. A global view of human selenium nutrition. Annu Rev Nutr 1987; 7: 227–50.
29. Hill HAO. Oxygen, oxidases and the essential trace metals. Philos Trans R Soc Lond Ser B 1981; 294: 119–28.
30. Grootveld M, Jain R. Recent advances in the development of a diagnostic test for irradiated foodstuffs. Free Radic Res Commun 1989; 6: 271–92.
31. Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Copper ion-dependent dam-

age to the bases in DNA in the presence of hydrogen peroxide. Biochemistry J 1991; 273: 601–04.
32. Minotti G, Aust SD. The role of iron in the initiation of lipid peroxidation. Chem Phys Lipids 1987; 44: 191–208.
33. Aruoma OI, Halliwell B, Laughton MJ, Quinlan JG, Gutteridge JMC. The mechanism of initiation of lipid peroxidation. Evidence against a requirement for an iron(II)-iron(III) complex. Biochem J 1989; 258: 617–20.
34. Gutteridge JMC. Lipid peroxidation: some problems and concepts. In: Halliwell B, ed. Oxygen radicals and tissue injury. Kansas: Allen Press, 1988: 9–19.
35. Haenen GRMM, Vermeulen NPE, Timmerman H, Bast A. Effect of thiols on lipid peroxidation in rat liver microsomes. Chem Biol Interact 1989; 71: 201–12.
36. Schoneich C, Asmus KD, Dillinger U, Bruchhausen F. Thiyl radical attack on polyunsaturated fatty acids: a possible route to lipid peroxidation. Biochem Biophys Res Commun 1989; 161: 113–20.
37. Halliwell B. Albumin, an important extracellular antioxidant? Biochem Pharmacol 1988; 37: 569–71.
38. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 1986; 246: 501–14.
39. Aruoma OI, Halliwell B. Superoxide-dependent and ascorbate-dependent formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Are lactoferrin and transferrin promoters of hydroxyl-radical generation? Biochem J 1987; 241: 273–8.
40. Grootveld M, Bell JD, Halliwell B, Aruoma OI, Bomford A, Sadler PJ. Non-transferrin-bound iron in plasma or serum from patients with idiopathic hemochromatosis. Characterization by high performance liquid chromatography and nuclear magnetic resonance spectroscopy. J Biol Chem 1989; 264: 4417–22.
41. McLaren GD, Muir WA, Kellermeyer RW. Iron overload disorders: natural history, pathogenesis, diagnosis and therapy. CRC Crit Rev Clin Lab Sci 1983; 19: 205–66.
42. McCay PB. Vitamin E: interactions with free radicals and ascorbate. Annu Rev Nutr 1985; 5: 323–40.
43. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 1989; 6: 67–75.
44. Wefers H, Sies H. The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. Eur J Biochem 1988; 174: 353-7.
45. Diplock AT, ed. Fat-soluble vitamins, their biochemistry and applications. London: Heinemann, 1985: 154–224.
46. Ingold KU, Burton GW, Foster DO, *et al.* A new vitamin E analogue more active than α-tocopherol in the rat curative myopathy bioassay. FEBS Lett 1986; 205: 117–20.
47. Gey KF, Brubacher GB, Stahelin HB. Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. Am J Clin Nutr 1987; 45: 1368–77.
48. Breimer LH. Ionizing radiation-induced mutagenesis. Br J Cancer 1988; 57: 6–18.
49. Marcillat O, Zhang Y, Lin SW, Davies KJA. Mitochondria contain a proteolytic system which can recognize and degrade oxidatively-denatured proteins. Biochem J 1988; 254: 677–83.
50. Muller DPR, Lloyd JK, Wolff OH. Vitamin E and neurological function. Lancet 1983; 1: 225–7.
51. Kretzer FL, Mehta RS, Johnson AT, Hunter DG, Brown ES, Hittner HM. Vitamin E protects against retinopathy of prematurity through action on spindle cells. Nature (Lond) 1984; 309: 793–5.
52. Halliwell B, Hoult JRS, Blake DR. Oxidants, inflammation and the anti-inflammatory drugs. FASEB J 1989; 2: 2867–73.
53. Baldwin SR, Simon RH, Grum CM, Ketai LH, Boxer LA, Devall LJ. Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet 1986; 1: 11–4.
54. Halliwell B, Aruoma OI, Mufti G, Bomford A. Bleomycin-detectable iron in serum from leukaemic patients before and after chemotherapy. Therapeutic implications for treatment with oxidant-generating drugs. FEBS Lett 1988; 241: 202–4.
55. Halliwell B, Gutteridge JMC. Oxygen radicals and the nervous system. Trends Neurosci 1985; 8: 22–6.
56. Hall ED, Yonkers PA. Attenuation of postischemic cerebral hypoperfusion by the 21-aminosteroid U74006F. Stroke 1988; 19: 340–4.
57. Hall ED. Effect of the 21-aminosteroid U74006F on post-traumatic spinal cord ischemia in cats. J Neurosurg 1988; 68: 462–5.
58. Hall ED, Yonkers PA, McCall JM, Braughler JM. Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg 1988; 68: 456–61.
59. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915–24.
60. Bolli R. Oxygen-derived free radicals and postischemic myocardial dysfunction ("stunned myocardium"). J Am Coll Cardiol 1988; 12: 239–49.
61. McCord JM. Oxygen-derived free radicals in post ischemic tissue injury. N Engl J Med 1985; 312: 159–63.
62. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. Lancet 1984; 1: 1396–8.